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Retina damage after exposure to UVA radiation on the early developmental stages of the Egyptian toad *Bufo regularis* Reuss

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ARTICLE INFO

Article history:
Received 11 February 2016
Received in revised form
19 April 2016
Accepted 16 May 2016
Available online 8 June 2016

Keywords:
Anura
UVA
Histology
Bufo regularis
Retina

ABSTRACT

The present study was carried out to investigate the histological and histochemical changes in the retina on different developmental stages of Egyptian toad *Bufo regularis*. Our experiment started when tadpoles begin to feed. The adapted embryos are divided into 3 large tanks of 200 embryos each, collections of samples started from feeding age every three days. Both histological and histochemical results showed that the general architecture of the retina organ is correlated with the state of development. Therefore, it displayed different characteristic features depending on the investigated developmental stage starting from the larval stage (feeding began, stage 44) and ending with the post-metamorphic stage 66. Also, the present work aimed to study the chronic effects of UVA on the retina structure of *B. regularis* during development and metamorphosis for the first time.

1. Introduction

The vertebrate eye develops three distinct embryological tissues (Akat & Arikan, 2013; Cvekl & Tamm, 2004; Fadool & Dowling, 2008; Trainor & Tam, 1995). During eye development, the initially undifferentiated, seemingly homogeneous, retinal progenitor cells develop into a layered array of seven cell types with different capabilities (Zhang et al., 2003). These include sensitive photoreceptor cells (bipolar interneurons), that transmit electrical stimulus from the photoreceptor to the ganglion cells and the ganglion cells, that transmit the information from the eye to the brain. The formation of these cell types, and their correct proportionality, is necessary for the proper function of the vertebrate eye (Zhang et al., 2003). In early embryological development the epidermis covering the eye is pigmented cells containing pigment granules derived from the egg (Balinsky, 1981).

Various histological studies were undertaken on retina (Link, Roth, & Rottluff, 1986; Vecino, Hernández, & García, 2004). At certain stage of embryonic development the cell of the retina undergo changes which results in a precise

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Peer review under responsibility of The Egyptian Society of Radiation Sciences and Applications.
http://dx.doi.org/10.1016/j.jrras.2016.05.006

specification of their projection on to the optic tectum (Stone, 1960). It has been shown by (Jacobson, 1968) that in *Xenopus laevis* these changes occur at embryonic stage 30–31, as tabulated by (Nieuwkoop & Faber, 1956). Dixon and Cronly-Dllon (1972) studied the fine structure of the developing retinal cells in *X. laevis* from stages 26–36 and they focused on the intercellular junctions between cells of the optic vesicle. The growth of retina in *X. laevis* by using auto radiographic methods was studied by (Straznicky & Gaze, 1971).

Amphibian populations are declining throughout the world (Blaustein & Kiesecker, 2002; Houlahan et al., 2000). More than 500 populations of frogs and salamanders are in decline and many are listed as of special conservation concern (Alford & Richards, 1999). In some regions, declines of amphibian populations appear to be greater than declines in other taxonomic groups (Pounds, Fogden, & Campbell, 1999). Numerous factors, including pathogens, introduced non-native species, UV radiation, contaminants, habitat destruction and global environmental changes are contributing to population declines in amphibians (Alford & Richards, 1999; Blaustein & Kiesecker, 2002; Blaustein, Romansic, Kiesecker, & Hatch, 2003).

The ultrastructure of the developing retina in many anuran has been extensively studied in *Rana pipiens* by Nilsson (1964); *X. laevis* by Fisher and Jacobson (1970), and Dixon and Cronly-Dllon (1972), however a study of the ultrastructure of the retina of the tadpole *Bufo regularis*, to our knowledge, has not yet been carried out. Also, the effect of ultraviolet radiation on the eye of the developing *B. regularis* has not been studied neither by light nor electron microscope. These studies were devoted to the photoreceptor cells as well as the retinal pigmented epithelia. As such we undertook this study with considerable interest, realizing that a better knowledge of its ultrastructure could be beneficial to other research workers, serving as a model for future experiments and studies on the retina.

2. Material and methods

2.1. *Sample collection*

This was described in details in previous study of the present author (Sayed, Elballouz, & Wassif, 2014).

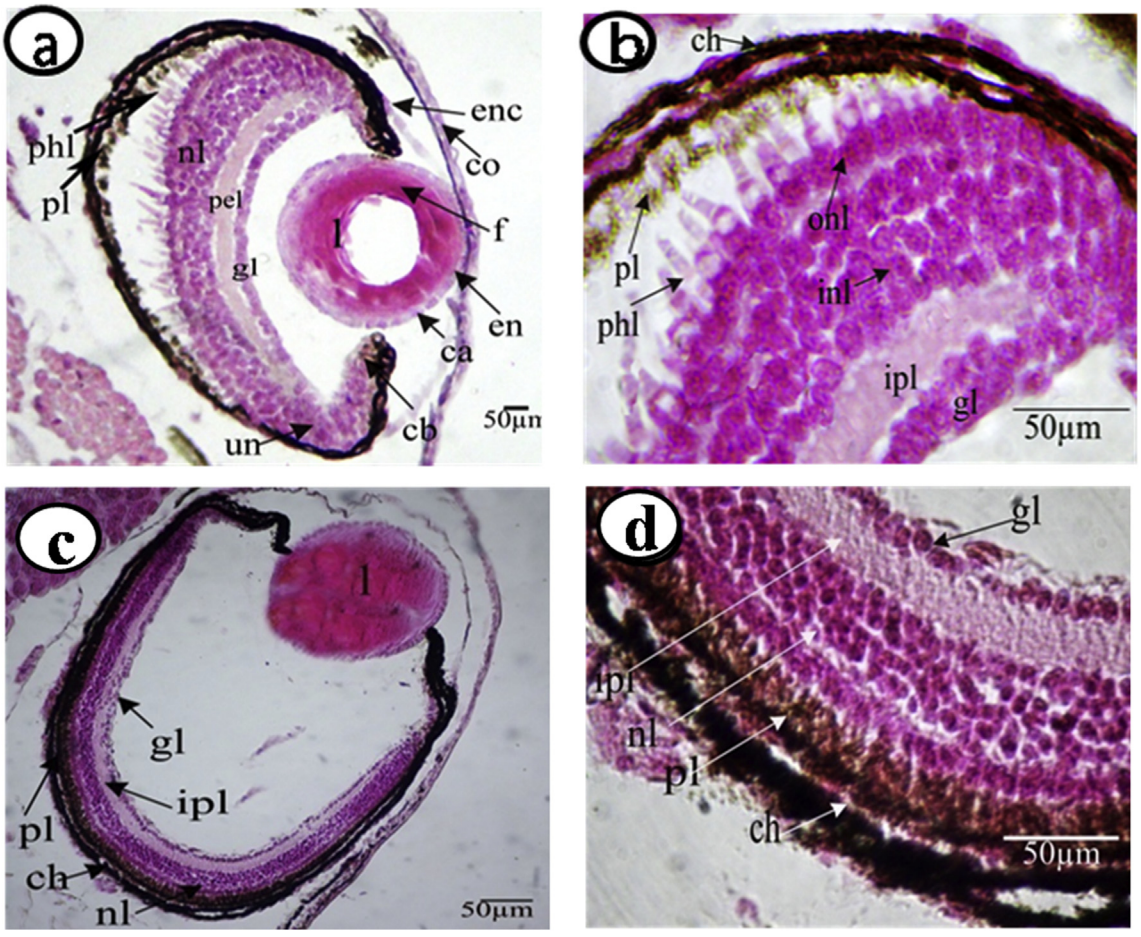

Fig. 1 – Sagittal sections of the eye of *Bufo regularis* tadpoles (a & b) stage 44, (c & d) stage 46 showing, choroid (ch), cornea (co), pigmented layer (pl), nuclear layer (nl), photoreceptor layer (phl), outer nuclear layer (onl), inner nuclear layer (inl), inner plexiform layer (ipl), ganglion cell layer (gl), lens (l), capsule (ca), endothelial cells (en), fibers (f), endothelial cell layer (enc), ciliary body (cb), undifferentiated cells (un). H & E, (a & c, X 100), (b & d, X 400) and scale bar = 50 µm.

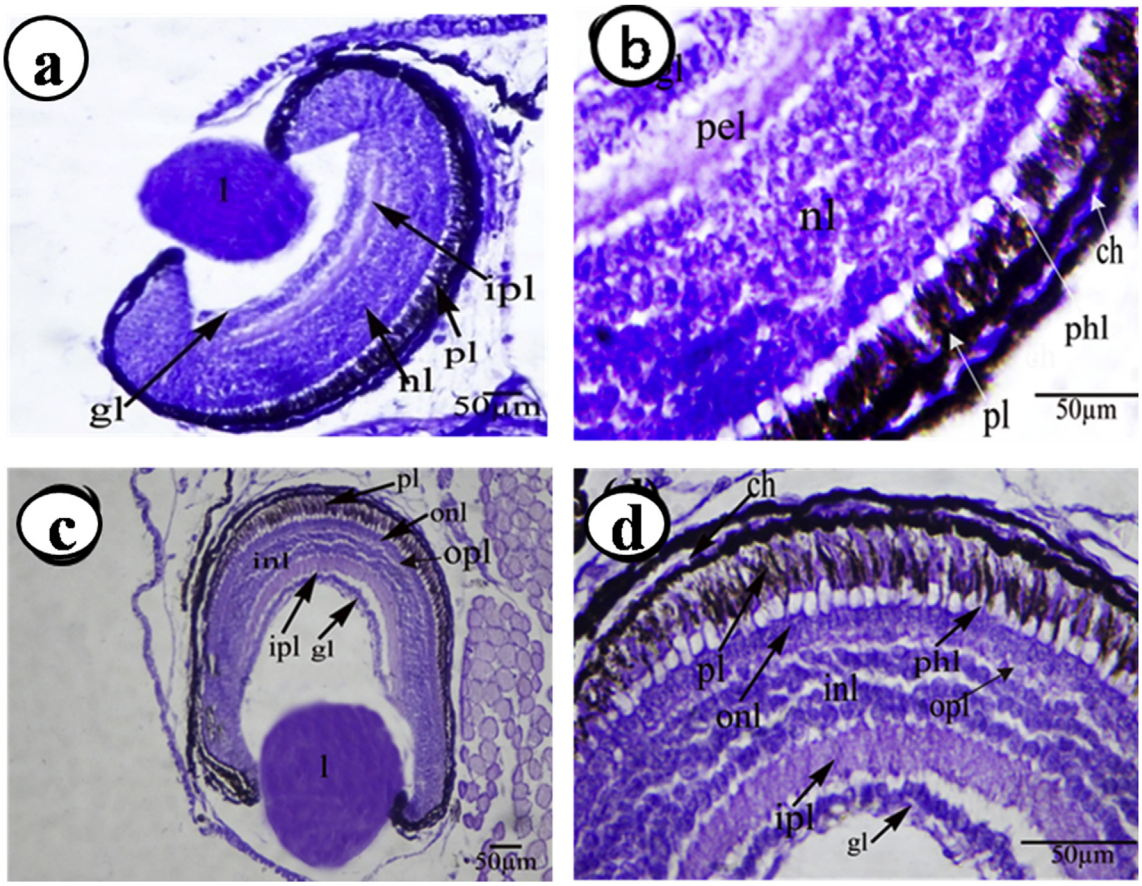

Fig. 2 – Sagittal sections of the eye of *Bufo regularis* tadpoles (a & b) stage 48, (c & d) stage 52 showing, choroid (ch), pigmented layer (pl), photoreceptor layer (phl), outer nuclear layer (onl), outer plexiform layer (opl), inner nuclear layer (inl), inner plexiform layer (ipl), nuclear layer (nl), plexiform layer (pel), ganglion cell layer (gl) and lens (l). H & E, (a & c, X100), (b & d, X 400) and scale bar = 50 µm).

2.2. *Experimental design*

The adapted embryos were subdivided into (4) large tanks (200 embryos per each) one tank of them for the UVA experiment. The conditions of the experiment were the same as that of acclimatization with daily changing all the tap water. Counting of the dead samples occurred every day to calculate the mortality rate. Twenty tadpoles were taken every three days during the experimental period for the different proposal studies. Some of tadpoles fixed in Bouin's or 10% buffered formalin, others fixed in Davidson solutions (33 ml of 95% ethanol alcohol, 25 ml of formalin, 11.5 ml of acetic acid and 33.5 ml of distil water). For Transmission Electron Microscope (TEM) tadpoles were fixed in 5% phosphate buffered (Ph 7.2) glutaraldehyde for 8-hours.

2.3. *UVA-exposure*

A foot paddle stage (stage 52) was chosen for the present study according to (Sedra & Micheal, 1961). Total length of the tadpole is 15.3–17.4 mm according to our investigation. Four animal groups, 50 individuals each: one control and three exposed to repeated doses of Ultraviolet-A (UVA) for 15 min, 30 min, and 60 min for 7 day at the same period of the day. Exposure took place in Petri dishes (14 cm in diameter and 2.5 cm height), three Petri dishes replicated for each group i. e 12 Petri dishes are used. UVA source was (A lamp of 366 nm, ULTRA-Violet products, ink. san cabrial, California U.S.A .model UVL-56) fitted at 10 cm above the Petri dish bottom (water level 2 cm) with changing the water every day. The specimens were left seven days without irradiation for recovery. After 14 days the irradiated and the control samples were dissected and the eye ball removed and fixed in Bouin's solution for light microscope preparations and 5% glutaraldehyde buffer solution for TEM preparations. No mortality observed during the present study.

2.4. *Histological, histochemical and TEM preparations*

This was described in details in previous study of the present author (Sayed, Elballouz, & Wassif, 2015).

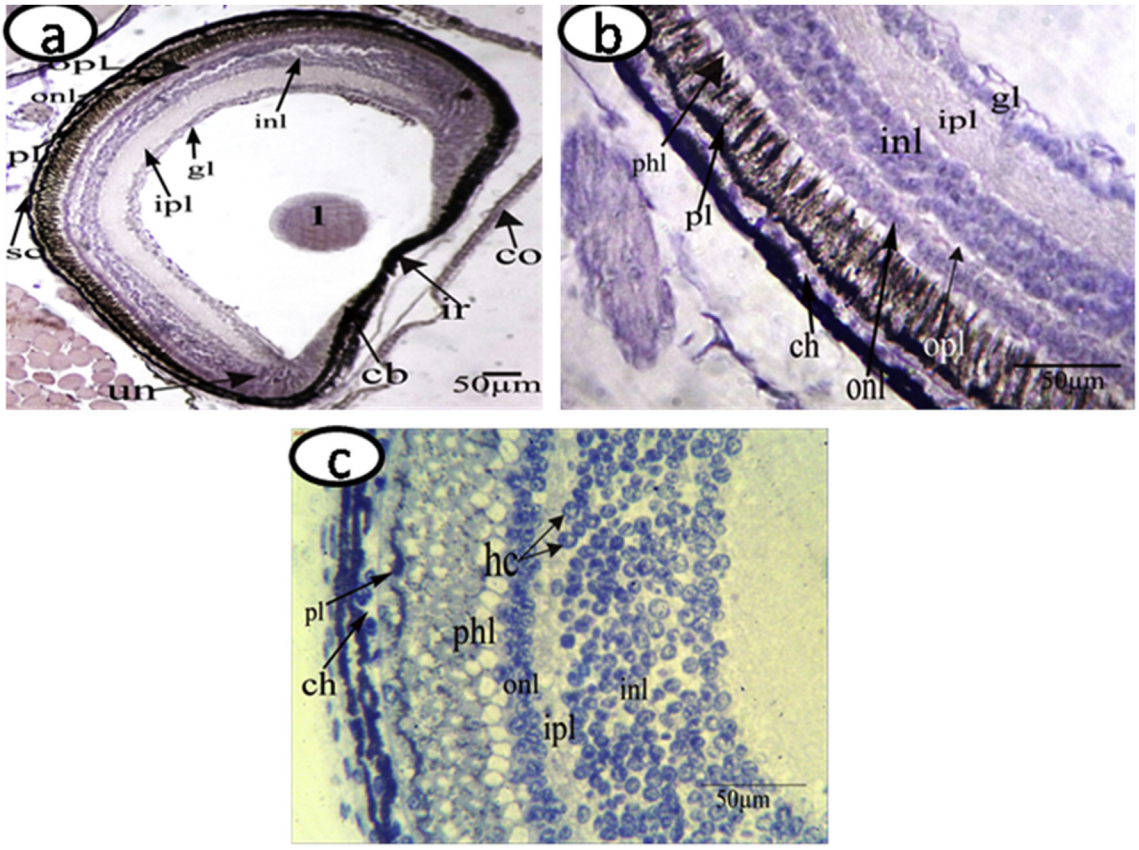

Fig. 3 – Sagittal sections (a & b) and tangential semithin section (c) of the eye of *Bufo regularis* tadpole (stage 54) showing, cornea (co), sclera (sc), choroid (ch), pigmented layer (pl), photoreceptor layer (phl), outer nuclear layer (onl), outer plexiform layer (opl), inner nuclear layer (inl), undifferentiated cells (un), inner plexiform layer (ipl), horizontal cells (hc), ganglion cell layer (gl), lens (l), ciliary body (cb) and iris (ir), H & E (a & b), Toluidine blue stain (c). (a, X100), (b & c, X400) and scale bar = 50 µm.

2.5. *Ethical statement*

All experiments were carried out in accordance with the Egyptian laws and University guidelines for the care of experimental animals. Procedures of the current experiment have been approved by the Committee of the Faculty of Science of Assiut University, Egypt.

3. Results

3.1. *A – General structure of the retina*

Developmentally the retina is formed of two layers of the invaginated optic cup; the outer layer is thinner and develops into the pigmented epithelium while the inner layer is a thicker and develops into the nervous tissues. The retina lies between the choroid externally and vitreous body internally (Burkitt, Young & Health, 1993). Histologically using light microscope the structure of the retina in the present study appears as (Figs. 1–7); the outer layer of the retina (pigmented layer) consists of low cuboidal pigmented epithelial cells forming a single layer. These cells were filed with a lot of melanin pigments which obscure the nucleus semithin sections clearly illustrated this layer (Fig. 3 c). This layer was rested on Bruch's membrane which separates them from the choroid. Their inner border sends cytoplasmic processes which extend to the outer segment of the next layer photoreceptor cells (rods and cones). The second layer is the nervous tissue of the retina which consists of the photoreceptor cells. These cells consisted of the outer, inner segments and the cell body which contains the nucleus. The third layer is the outer nuclear layer which contains one row of the nuclei of the photoreceptor cells. The fourth layer is the outer plexiform layer. This layer consisted of terminal processes of the photoreceptor cells with the dendrites of the bipolar nerve cells. The fifth layer is the inner nuclear layer (bipolar nerve cells). The sixth one is the inner plexiform layer; this layer is thicker than that of the outer one. The seventh layer is the nuclei of the ganglionic layer of the neurons. The inner plexiform layer consisted of

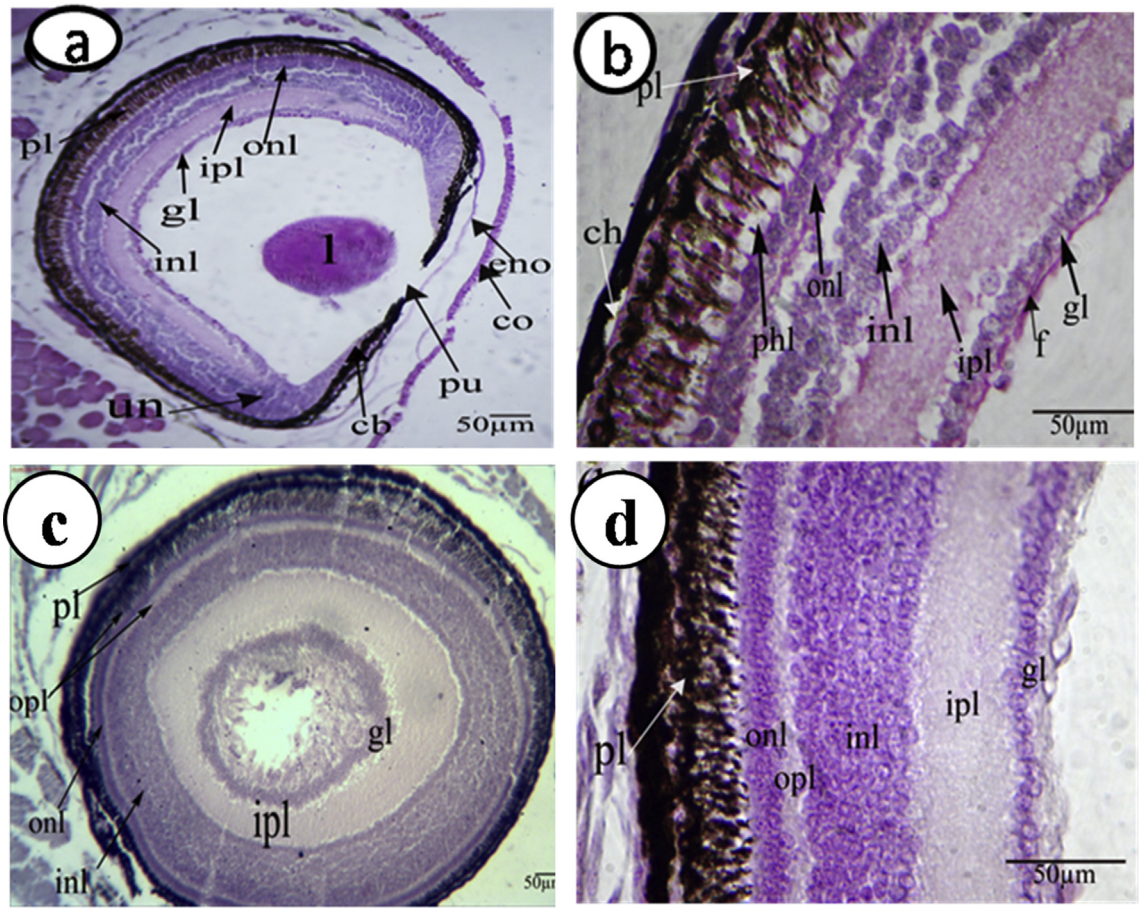

Fig. 4 – Sagittal sections (a & b) and tangential section (c & d) of the eye of *Bufo regularis* tadpoles showing, (a & b) stage 55, (c & d) stage 64, choroid (ch), endothelial cells layer (eno), pigmented layer (pl), photoreceptor layer (phl), outer nuclear layer (onl), inner nuclear layer (inl), undifferentiated cells (un), inner plexiform layer (ipl), ganglion cell layer (gl), nerve fibers (f), lens (l), ciliary body (cb), pupil (pu). H& E (a & c, X100), (b & d, X400) and scale bar = 50 µm.

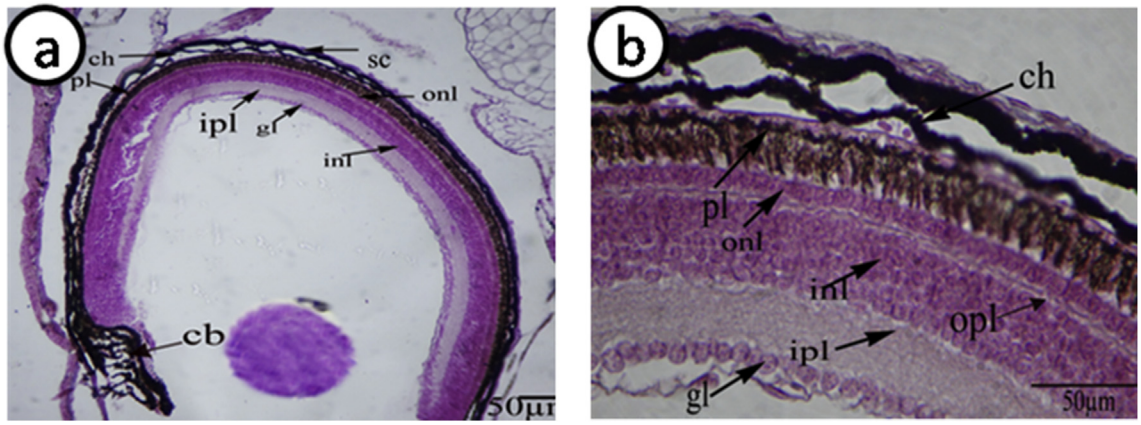

Fig. 5 – Sagittal sections of the eye of *Bufo regularis* tadpoles showing, (a & b) stage 66, sclera (sc), choroid (ch), pigmented layer (pl), outer nuclear layer (onl), outer plexiform layer (opl), inner nuclear layer (inl), inner plexiform layer (ipl), ganglion cell layer (gl) and ciliary body (cb), H & E, (a, 0X100), (b, X400) and scale bar = 50 µm.

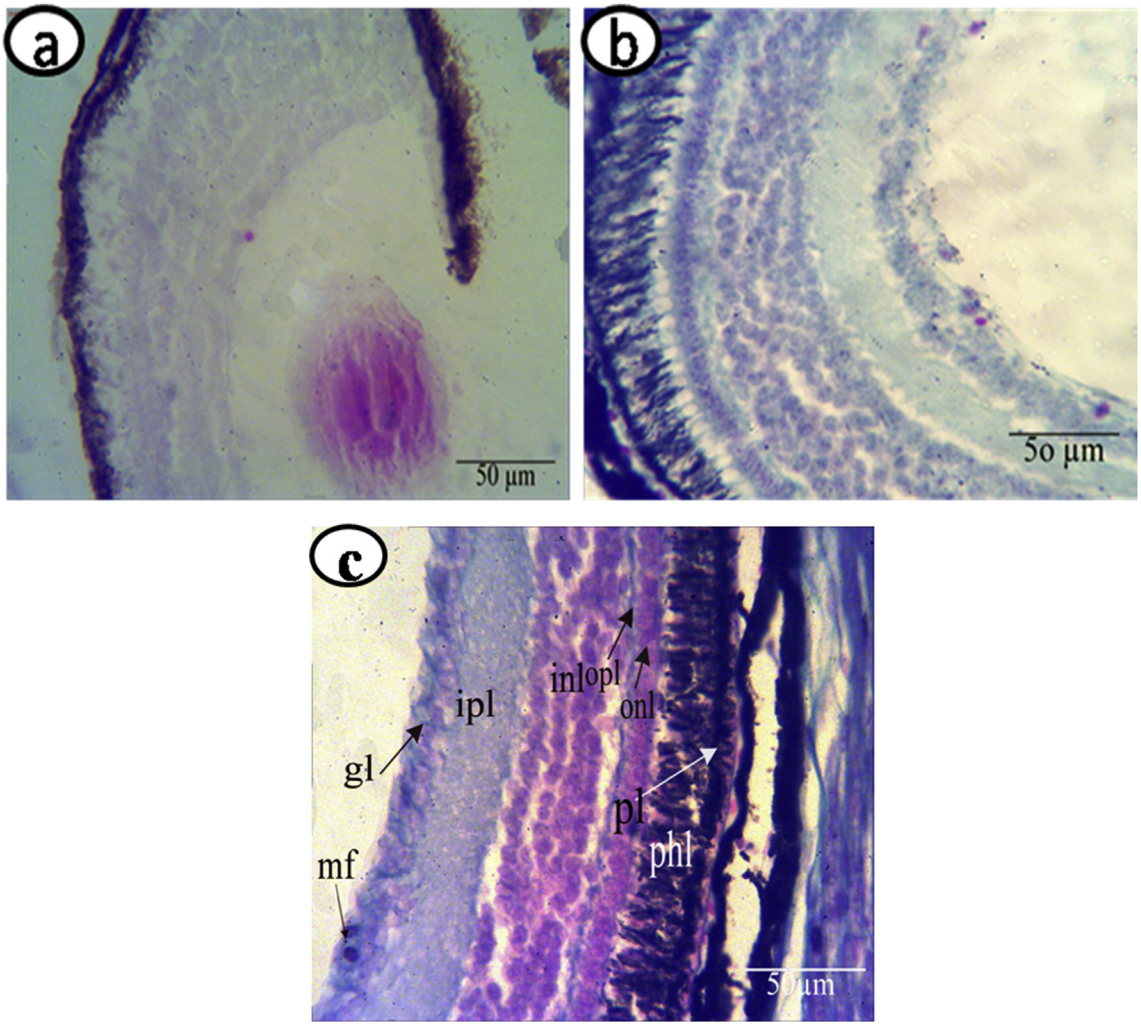

Fig. 6 – Sections of the eye of *Bufo regularis* tadpoles showing, (a) stage 44, (b) stage 54, (c) stage 66, negatively stained in (a) increase in the intensity of stain as development progresses (b & c), pigmented layer (pl), photoreceptor layer (phl), outer nuclear layer (onl), outer plexiform layer (opl), inner nuclear layer (inl), inner plexiform layer (ipl), ganglion cells layer (gl) and mitotic figure (mf), (Milligan's trichrome stain, X 400 and scale bar = 50 µm).

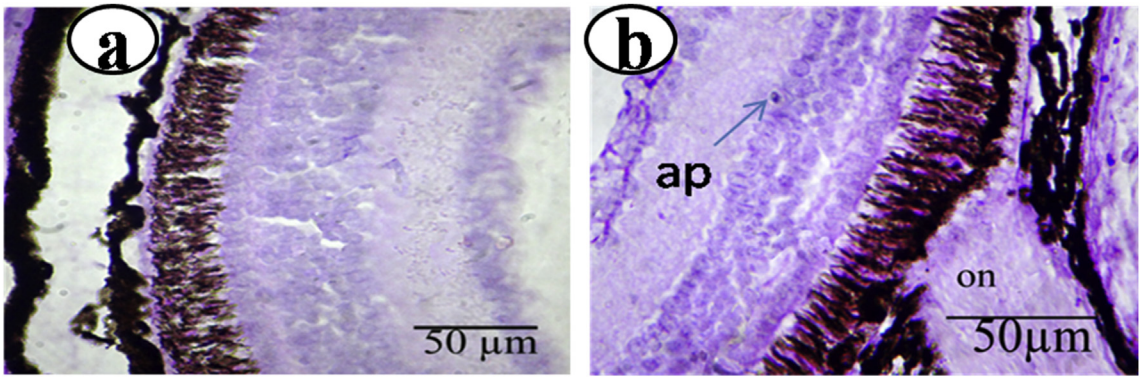

Fig. 7 – Sagittal sections of the eye of *Bufo regularis* tadpole (stage 66) showing, a very small amount of glycogen in the retinal layers, apoptotic nuclei (ap), optic nerve (on), (a, PAS and H without diastase enzyme), (b, PAS and H with diastase enzyme), X 400 and scale bar = 50 µm.

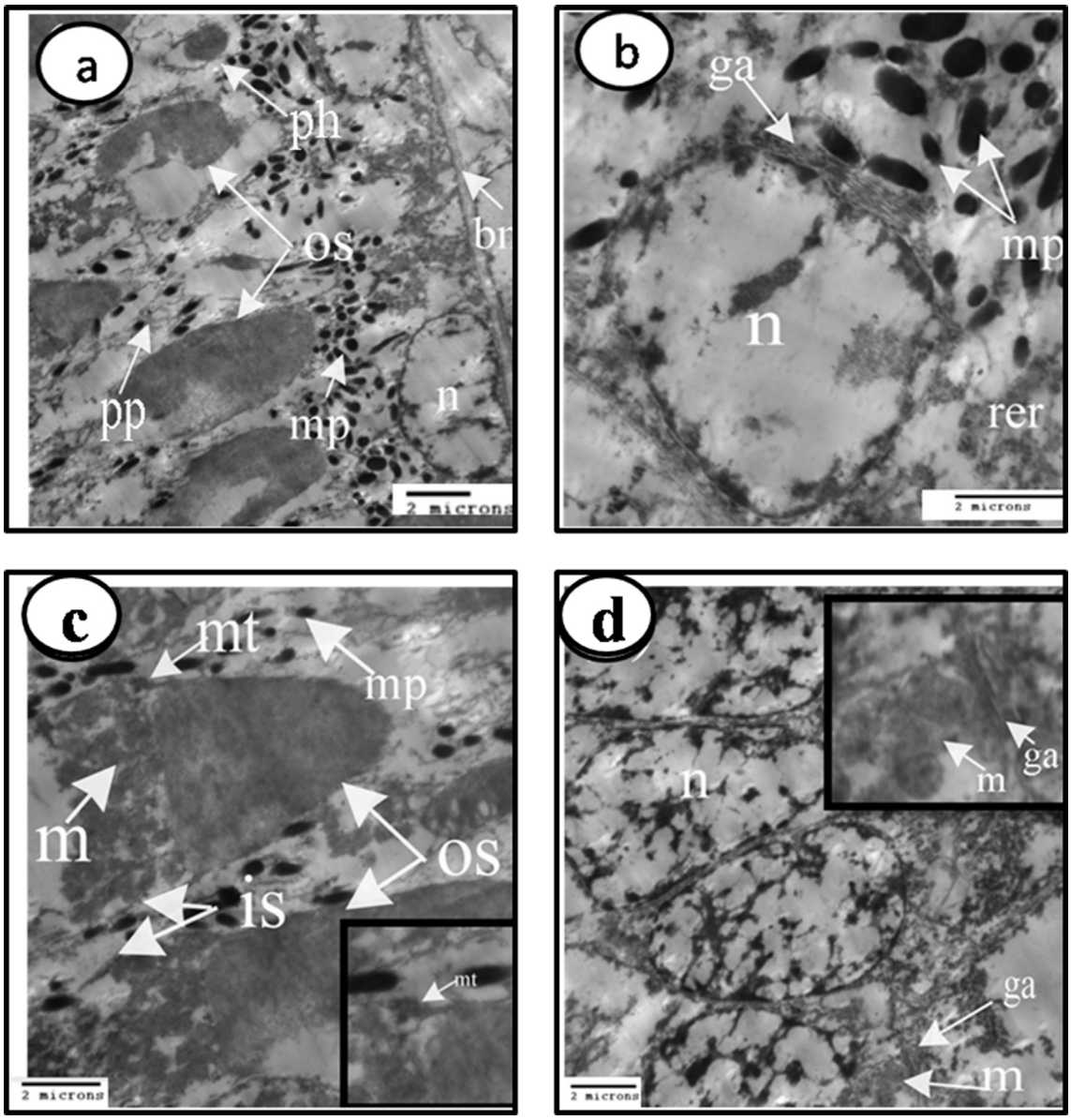

Fig. 8 – Transmission electron micrograph of longitudinal sagittal sections through the control retina layers of *B. regularis* showing: a) The retinal pigmented cell with its basally located nucleus (n). Melanin pigments (mp) fill the inner portion of the cell. The outer segment (os) of the photoreceptor cells surrounded by the processes of the pigment cells (pp) and phagosome (ph). Also showing Bruch's membrane (bm), (X 3600 and scale bar 2 μ). b) Pigmented cells showing, Golgi apparatus (ga), euchromatin in nucleus (n), outer segment (os), rough endoplasmic reticulum (rer) at the vicinity of the nucleus and different sizes and shapes of melanin pigments (mp), (X10000 and scale bar 2 μ). c) The outer segments (os) contain a stack of flattened membranous discs and an inner segment (is) surrounded by the processes of the pigment cells (pp), the inner segment (is) filled by rounded mitochondria (m), in situ magnified portion showing microtubule (mt), (X 5900 and scale bar 2 μ). d) The body of the photoreceptor cells showing, nucleus (n) surrounded by Golgi apparatus (ga), inner segment (is), mitochondria (m), nucleus (n), outer segment (os), rough endoplasmic reticulum (rer), In situ, magnified portion showing mitochondria and Golgi apparatus, (X 5800 and scale bar 2 μ).

the synapses of the axons of the bipolar neurons with the dendrites of the ganglionic cells. The axon of these ganglion cells forms the optic nerve.

The lens substance at this stage consists of many nucleated fibers; the cell membranes of adjacent fibers are fused. The anterior lens surface is covered by a single layer of low cubical cells which retain their nuclei, this layer merger with the residual proliferative cells at the equatorial margin of the lens (Fig. 1 a).

As development of the tadpoles' progress, the eye diameters, the cell numbers and the thickness of the retinal layers increased.

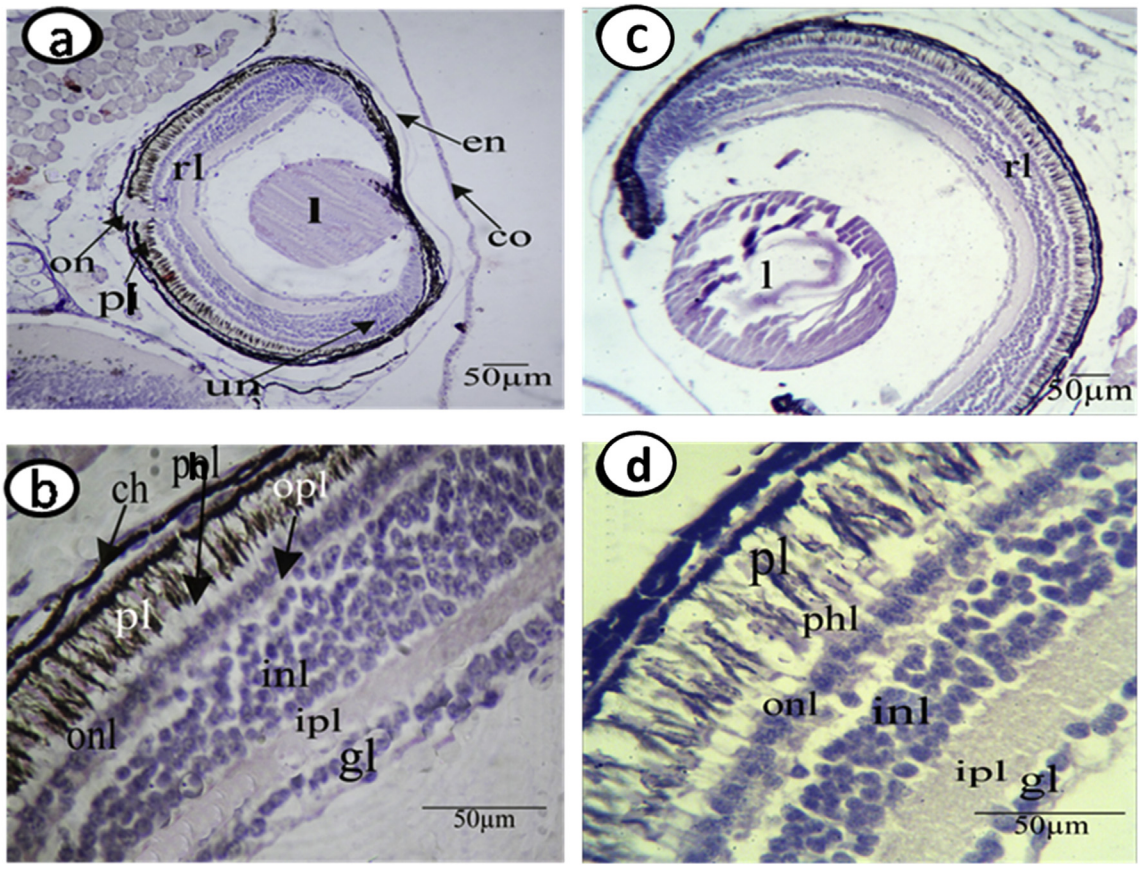

Fig. 9 – Sagittal section of the eye in the stage 58 b of *Bufo regularis* control. (a & b) section through the tadpoles eye of control showing, choroid (ch), cornea (co), endothelial cell layer (en), pigmented layer (pl), optic nerve (on), photoreceptor layer (phl), outer nuclear layer (onl), outer plexiform layer (opl), inner nuclear layer (inl), inner plexiform layer (ipl), ganglion layer (gl), retinal layers (rl), lens (l), undifferentiated cells (un), (c & d) Histological lesions of the eye in the stage 58 b of *Bufo regularis* embryos after exposure to 15 min UVA (366 nm) showing, sever damage in the lens and partially in the photoreceptor cells, choroid (ch), pigmented layer (pl), photoreceptor layer (phl), outer nuclear layer (onl), inner nuclear layer (inl), inner plexiform layer (ipl), ganglion layer (gl), retinal layers damaged (rl) and lens (l) H & E (a, X 100), (b, X 400) scale bar = 50 µm.

At stage 44 the outer plexiform layer was too thin to be distinguished. The thickness of this layer increased as development progressed (Figs. 1–5).

Staining with Milligan's trichrome stain showed the increment in the intensity of the stain of the retinal layers as the stage of the tadpoles progressed (Fig. 6).

Staining with Schiff's reaction (PAS) revealed that there was a moderate amount of glycogen as verified by staining the control sections with diastase enzyme where glycogen removed from the section (Fig. 7).

3.2. B – *Ultrastructure of the retinal layers*

Ultrastructurally, the pigmented layer of the retina at this stage consisted of low cuboidal cells with basally located more or less rounded euchromatin nuclei. The pigmented epithelial cells rested on the basement membrane. There were no junctions between the basal lamina and the pigmented epithelial cells. These cells contain a plenty of free ribosomes, polysomes as well as rough endoplasmic reticulum and melanin pigments which present in the inner part of the cell. The inner border of these cells sends cytoplasmic processes which extended to touch the tips of the photoreceptor cells of the next layer. Melanin pigments were seen distributed within these processes.

Well-developed Golgi apparatus was noticed at the vicinity of the nucleus of the pigmented epithelial cells, but mitochondria rarely found. The choroid is a layer of loose vascular supporting tissue lying between the sclera externally and the retina internally. The choroid contains numerous large, heavily pigmented melanocytes. The choroid and retina are separated by Bruch's membrane (Fig. 8).

The outer segments of photoreceptor cells have a regularly cylindrical shape and contain a stack of flattened transverse membranous discs which incorporate the photosensitive pigment rhodopsin. The inner segments of the photoreceptor cells contain Golgi apparatus, multiple numbers of mitochondria, polysomes, RER and microtubules of the cilium. The inner segment joints the cell body which contains the nucleus. The cell body sends its central process to synapse with

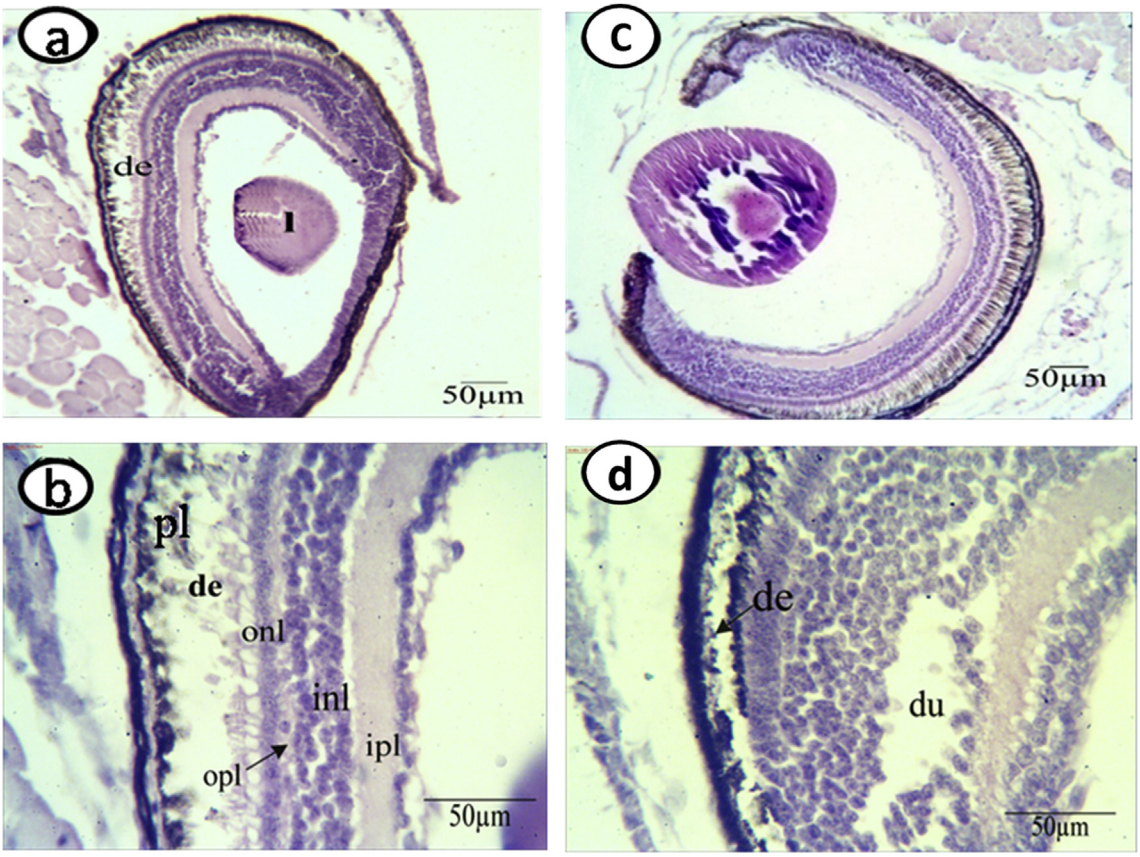

Fig. 10 – Histological lesions of eye in the stage 58 b of *Bufo regularis* embryos, (a & b) after exposure to 30 min UVA (366 nm) showing, decrease in the diameter of the eye in (section, a) and sever damage in the lenses (l), degeneration in the pigmented layer (de) and in the outer and inner segments of the photoreceptor cells, pigmented layer (pl), outer nuclear layer (onl), outer plexiform layer (opl), inner nuclear layer (inl), inner plexiform layer (ipl) and lens (l), (c & d) after exposure to 60 min UVA (366 nm) showing, damage in Bruch's membrane (de) as well as degeneration in the undifferentiated cells at the lateral side of the retina (du) and great damage in the lens, H&E (a, X 100), (b, X 400) scale bar = 50 µm.

peripheral processes of the bipolar nerve cell (outer plexiform layer) (Fig. 8).

3.3. C – *Histopathological changes of the retina after exposure to UVA (366 nm)*

3.3.1. A – *Light microscopy*

After the exposure of tadpoles to UVA for 15, 30 and 60 min daily for seven days, then let the tadpoles seven days for recovering, partially injury of the outer segments of the photoreceptor cells and damage in the lens was noticed after 15 min (Fig. 9 c & d), damage in the pigmented layer was observed after 30 min (Fig. 10 a & b) and damage in the undifferentiated nuclear layers in the lateral sides of the retina was occurred after 30 min and 60 min (Fig. 10). Ultraviolet exposure has been reported to have harmful effects on the cornea, the lens and the retina (Figs. 9 c, d and 10) compared with control tadpoles (Fig. 9 a, b).

3.3.2. B – *Ultrastructure of the pigmented layer and photoreceptor cells of the retina*

The damage of the retina observed by the light microscope (Figs. 9 and 10) was authenticated by the transmission electron microscope in the form of malformation in the outer segments of the cone cells, disintegration in the outer segment of the rod cells and an increase in the numbers and distribution of Golgi apparatus. Decreasing in the number of mitochondria and disturbance in their distribution in the inner segments (Figs. 11–13) was notice in comparing with the control (Fig. 8). The damage of the photoreceptor cells increased with the time of exposures. Complete damage of the outer segments of the rods and cones was occurred after 60 min exposure (Fig. 13). The present findings emphasize the sensitivity of the photoreceptor cells to UVA.

4. Discussion

Our findings in the control group were in agreement with the findings of (Braeckevelt, 1992, 1993, 1998; Braeckvelt, 1983; Young, 1968), where the structures of the outer segments of the *B. regularis* tadpole retina as revealed by electron microscopy in the current work were similar to findings in various species. Whereas, the two classes of visual cells, rods and cones, are distinguished by differences in the shape of the

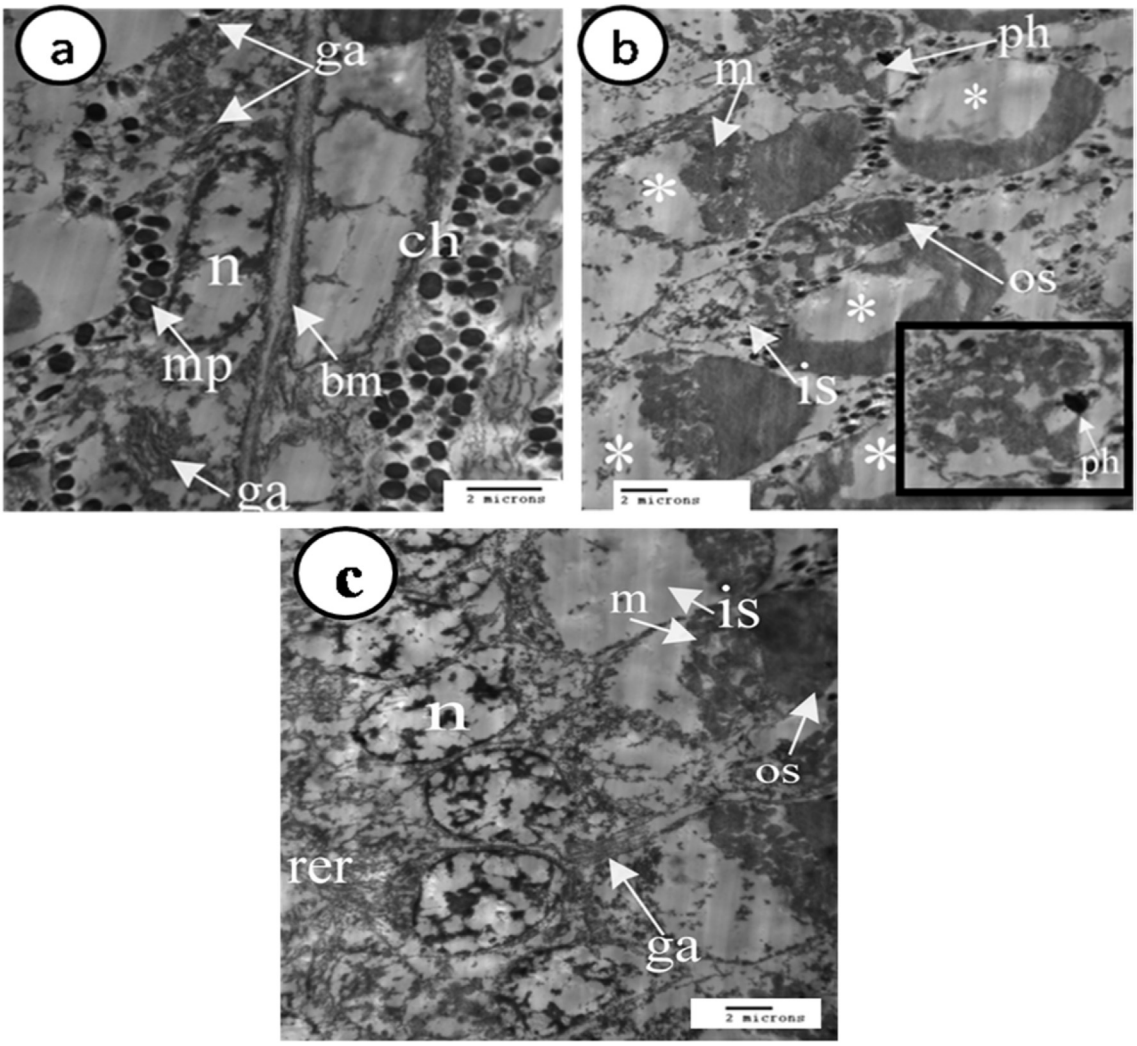

Fig. 11 – Transmission electron micrograph of retinal layers of *B. regularis* after exposure to 15 min UVA (366 nm) showing: (a) Pigmented epithelial layer showing, Bruch's membrane (bm), choroid (ch), Golgi apparatus (ga), melanin pigments (mp), heterochromatin in nucleas (n), (X 3600 and scale bar 2 μ). (b) Photoreceptor cells showing, mitochondria (m), nucleus (n), outer segment of photoreceptor layer (os), malformation in outer segment of photoreceptor layer (*), in situ magnified portion showing phagosome (ph), (X 3600 and scale bar 2 μ). (c) Nuclear layer showing, Golgi apparatus (ga), inner segment (is), mitochondria (m), nucleus (n), outer segment (os), rough endoplasmic reticulum (rer), (X 3600 and scale bar 2 μ).

outer segment and in rods the outer segment is cylindrical but in cones it is conical or tapered. This compact, repetitive arrangement serves to concentrate, stabilize, and orients the photosensitive molecules which are the structural components of the discs (Young, 1971).

In the present investigation, observations by light and transmission electron microscopy revealed that in control group, the structure of the retinal pigmented layer in *B. regularis* tadpoles consisted of a single layer of low cuboidal epithelium and numerous apical processes.

The retinal pigment epithelium (RPE) in some domestic animals consisted of one row of cuboidal cells (Braeckevelt, 1984, 1990; Braeckvelt, 1985, 1986, 1988; Esfandiari, Gholami, & Safavi, 2008; Kuwabara, 1979 58–82; Nguyen-Legros, 1978). In red kangaroo (*Macropus rufus*) the retinal epithelium consisted of a single layer of pigmented squamous cells (Young & Braekevelt, 1993). The basal portions of the RPE shows numerous very deep basal (scleral) infoldings and extensive apical (vitreal) processes which enclose photoreceptor outer segments (Braeckevelt, 1990; Young & Braekevelt, 1993). In the present work the basal infoldings did not found. The presence of highly infolded basal epithelial membranes is detected to be indicative of a heavy involvement in transport and this function is well established for the retinal epithelium (Steinberg & Miller, 1973). These basal infoldings are deeper in avian species than is normally reported for mammalian species and may be necessary to compensate for the less-well fenestrated choriocapillary endothelium noted in birds (Braeckevelt, 1984).

In the present work a large vesicular euchromatin nucleus was noticed in the control group while in UVA exposure groups the cell nucleus became more heterochromatin in comparison with the control group. Well-developed Golgi apparatus were seen in the pigment cell of the control group

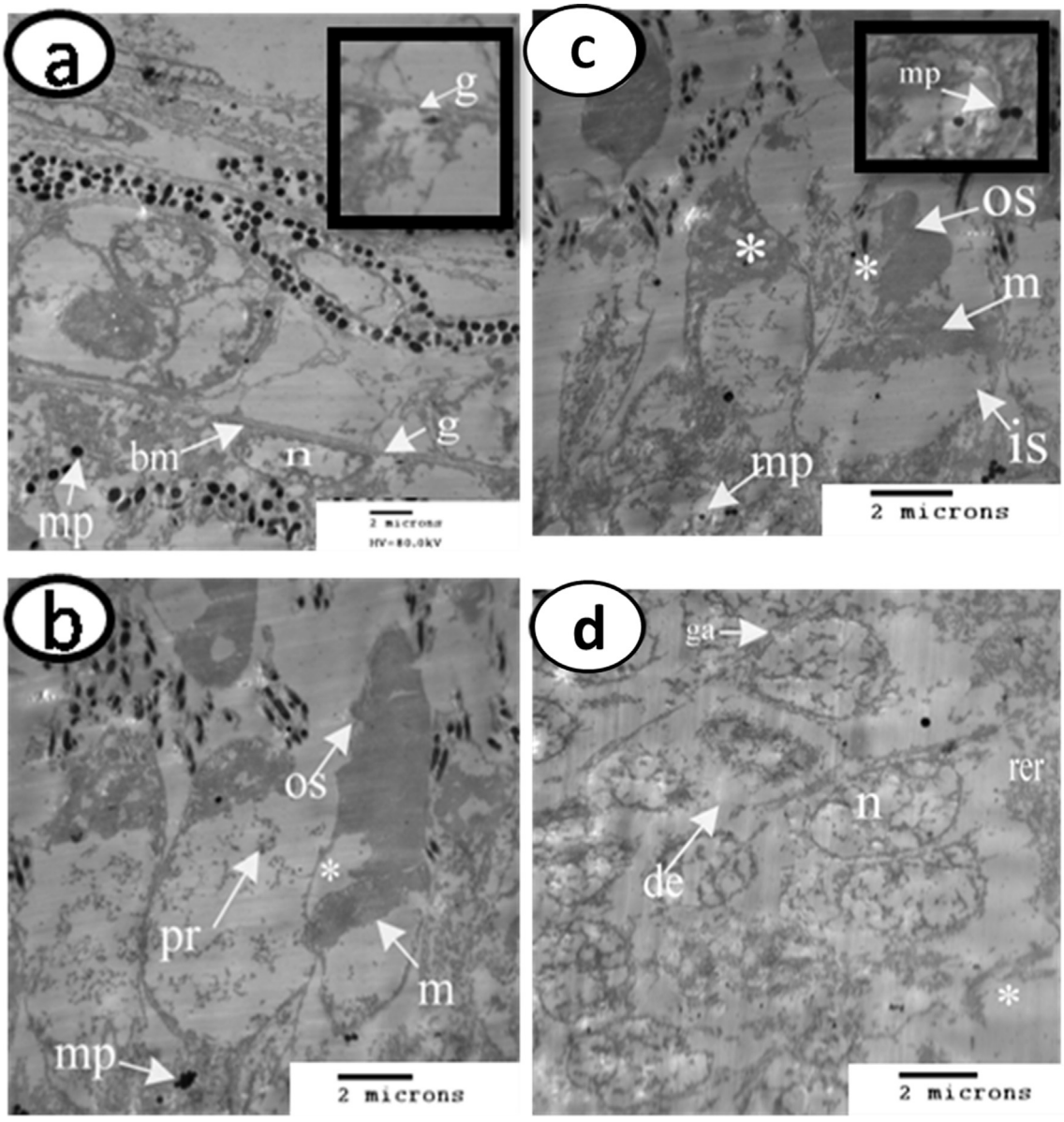

Fig. 12 – Transmission electron micrograph of longitudinal sagittal sections through the retina layers of *B. regularis* after exposure for 30 min to UVA (366 nm). a) Pigmented epithelial cells showing, gap in Bruch's membrane (g), migrating melanin pigments (mp) around the outer segments, heterochromatin in nucleus (n), (X 3600 and scale bar 2 μ). b) Photoreceptor cells showing, gradually decrease in the number and distribution of mitochondria (m), melanin pigments (mp), sever damage (*) in the outer segment of photoreceptor cells (os), polyribosome (pr), (X 3700 and scale bar 2 μ). c) Photoreceptor cells showing, decrease in the number and distribution of mitochondria (m), melanin pigments (mp), sever damage (*) in the outer segment of photoreceptor cells (os), inner segment of photoreceptor layer (is), in situ magnified portion showing, melanosome at the vicinity of the nucleus, (X 3600 and scale bar 2 μ). d) Nuclear layer showing, degeneration (de) in some cells in the inner nuclear layer, and an increase in the space between nuclear layer and the outer plexiform layer (*), Golgi apparatus (ga), nucleus (n), rough endoplasmic reticulum (rer), (X 3600 and scale bar 2 μ).

where more than one set of Golgi apparatus were seen in the retinal pigmented epithelium cell in the tadpoles exposed to UVA especially after 15 min. These changes may be due to the great activity in this cell in synthesis melanosomes to avoid the action of UVA. RER and polyribosomes are seen in this cell, all indicative of metabolically very active cells. Yousofi, Esfandiari, and Bozorgi (2009) found that in control group, the structure of the retinal pigmented layer in cat was similar to other mammals, while in light exposure group, the cell nucleus became more euchromatin in comparison with control group. These changes were due to increased activity of retinal pigmented cells (Banks, 1993).

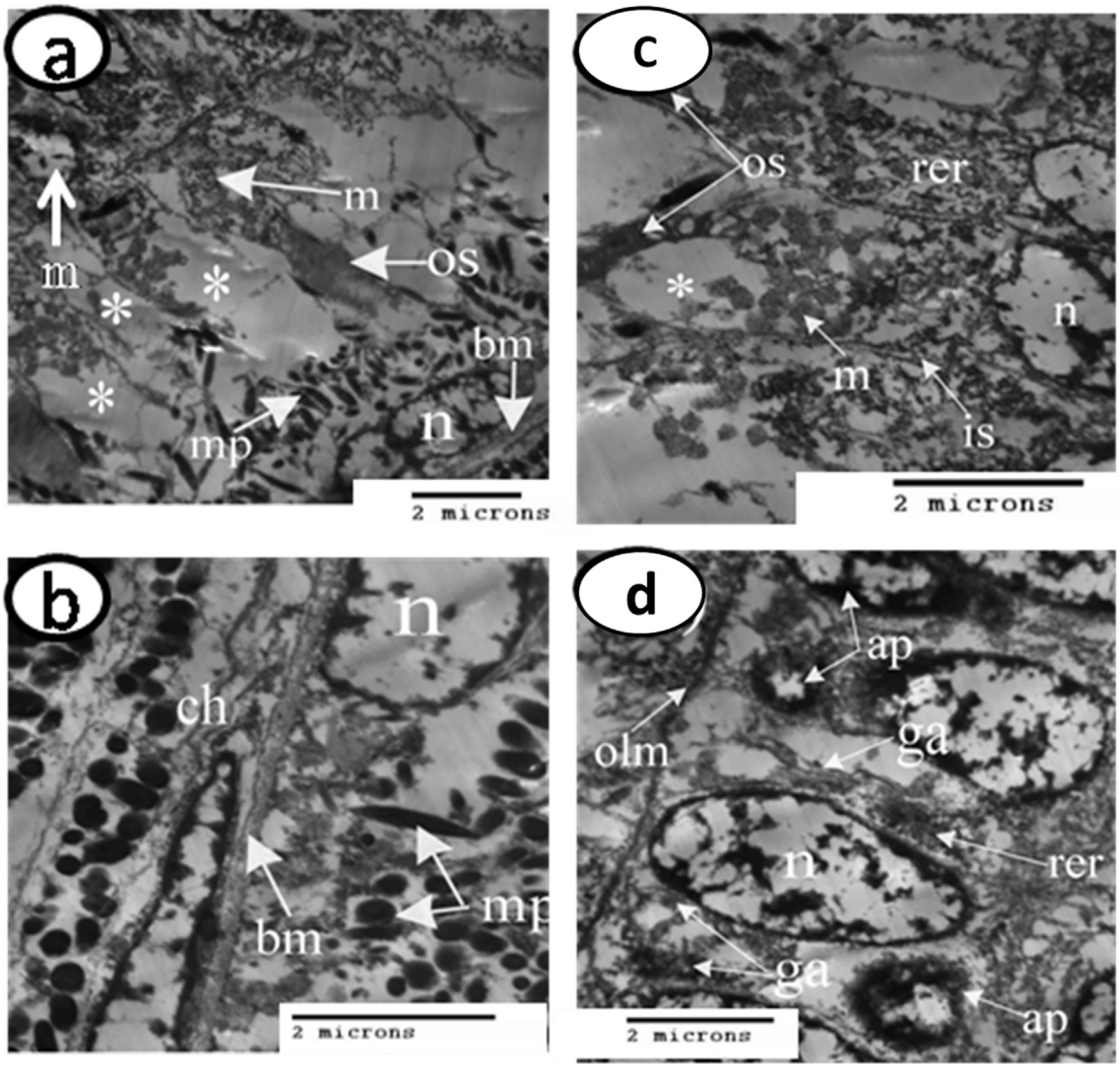

Fig. 13 – Transmission electron micrograph of retinal layers of *B. regularis* tadpoles after exposure to 60 min UVA (366 nm). (a & b) Photoreceptor cells showing, Bruch's membrane (bm), choroid (ch), inner segment of photoreceptor layer (is), mitochondria (m), melanin pigments with different size of melanophores (mp), nucleus (n), outer segment of photoreceptor layer (os), rough endoplasmic reticulum (rer), malformation in the photoreceptor cells (*). (a, X 4800. b, X 10000 and scale bar 2 μ). c) Photoreceptor cells showing, Bruch's membrane (bm) mitochondria (m), melanin pigments (mp), nucleus (n), outer segment of photoreceptor layer (os), rough endoplasmic reticulum (rer), intercellular space (is), malformation in the inner segment of photoreceptor cells (*), (X 7200 and scale bar 2 μ). d) Outer nuclear layer showing, apoptotic nuclei (ap), Golgi apparatus (ga), nucleus (n), outer limiting membrane (olm), rough endoplasmic reticulum (rer), (X 7200 and scale bar 2 μ).

In the present work few phagosomes of outer segment material was noted within the tadpoles RPE cells. Such phenomenon was noticed in light-adapted mallard by (Braeckevelt, 1990). The few phagosomes of outer segment material noted within the RPE cells of the light-adapted mallard are presumably the remains of the burst of rod outer segment shedding which is known to occur soon after the onset of light (Young, 1978; Young & Bok, 1979).

In the present investigation melanin pigments noticed to be located within the apical processes of these cells. The melanosomes of the retinal epithelial cells of the mallard are in the light-adapted state, located almost exclusively within the apical processes of these cells. The location of melanosomes only within the apical processes of the RPE cells in light-adaptation may be explained by (Meyer, 1977) observation that the photomechanical changes in birds are quite rapid and extensive.

At stage 44 the outer plexiform layer (OPL) was noticed too thin to be distinguished. The thickness of this layer increased as development progressed. Craft, Fulton, Silver, and Albert (1982) mentioned that the outer plexiform layer (OPL) appeared at 4–5 days after birth in albino rat and separates an

outer nuclear layer (ONL) from the inner nuclear layer (INL). The growth of retina in *X. laevis* by using auto radiographic methods was studied by (Straznicky & Gaze, 1971). They showed that DNA synthesis occurs after the period of specification in the cells at the periphery of the retina. In this region cell division takes place well beyond stage 31, as tabulated by (Nieuwkoop & Faber, 1956), in order to increase the dimensions of the developing retina. Retinal cell numbers increased with tadpole growth (McDiarmid & Altig, 1999). These results are in agreement with the present studies, where we noticed cell divisions occurred at the periphery of the retina (undifferentiated portion of the retina).

The retina negatively stained by Milligan's trichrome stain at stage 44 and the density of staining increased as development of tadpoles progressed. The investigators suggested that the increment in the density of staining may be due to a production of new proteins which increased as the development of the tadpoles progressed. This suggestion is in agreements with the analysis of proteins by electrophoresis (SDS-PAGE) and DNA electrophoresis analysis (RAPD-PCR) where new bands appeared and the thickness of the bands increased, respectively, as the development of the tadpole progressed (Sayed et al. 2014).

In the present work the investigators found a positive substance saliva-insoluble PAS in tadpole retina. Such substance has been reported as a glycolipo-protein complex found in the outer segments (Lillie, 1952). However, there are few studies documenting population changes due to exposure to UV radiation (Sayed, 2015) and contaminants. Long-term studies, preferably with experimental components, on the effects of multiple stressors are necessary to understand more fully how amphibian populations are affected so that conservation efforts can be implemented to ameliorate the problem (Blaustein et al., 2003). In the present investigation histopathological changes of the retina of *B. regularis* tadpole were recorded and the degree of damage increased in corresponding with increasing the time of exposure to UVA.

Using light microscope, the damage of the retinal layers were recorded in the photoreceptor cells, in some extend in pigmented layer and in the undifferentiated portions at the lateral sides of the retina. These damages were authenticated by the transmission electron microscope in the present investigation. The greatest damage recorded in the photoreceptor cells where the damage increased with the time of exposure and these damages irreversibly because the tadpoles were examined after recovery.

In the present work we noticed decrease in the number of mitochondria and disordered their distributions. Lawwill (1982) observed damage in mitochondria in monkey retinas after irradiation at various visible wavelengths. He concluded that mitochondria are the structure most sensitive to anatomic change.

Moreover, at the ultrastructural level the most prominent finding in the present work is the severe swelling of the mitochondria, which is in accord with the histologic appearance of UVA phototoxicity in primates (Rapp, Tolman, & Dhindsa, 1990) and their results supported the hypothesis of mitochondrial enzymes are the most likely mediators of UVA damage. Many studies using different animals models were reported the damage in the photoreceptor cells after threshold doses of UVA (Collier, Waldron, & Zigman, 1989; Rapp & Smith, 1992).

In the present study we noticed the migration of melanin pigments towards the outer segments of the photoreceptor cells and more than one set of Golgi apparatus were observed near the condensed heterochromatin nucleus of the pigmented cell after the exposure of the retina of tadpole to UV 366 nm for 15 min. Those results were in agreement with other studies using the same dose of UVA in monkey (Ham, Mueller, Ruffolo, Guerry, & Guerry, 1982) and *Procambarus clarkii* (El-Bakary & Sayed, 2011).

The cell membranes of the pigmented epithelial (PE) and rod outer segment (ROS) were severely damaged by UV irradiation indicating the lipid peroxidation level increase after UVA (Feeney & Berman, 1976; Mekkawy, Mahmoud, Osman, & Sayed, 2010).

5. Conclusion

It could be suggested that the amphibia metamorphosis of *B. regularis* caused severe changes to some organs such as eye. Also, the present findings emphasized that the chronic exposure of tadpoles of the Egyptian toad to some doses of UV-A caused chronic damage to the retina especially to photoreceptor cells.

Acknowledgments

This research funded by Assiut University and the Ministry Higher Education from Libyan Government.

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